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A picture can speak a thousand words; the strengths and weaknesses of collagen fiber orientation analysis methods in skin fibrosis

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Introduction

Skin fibrosis or scarring secondary to injury leads to significant patient morbidity through physical disability and psychological stress. Advances in scar management have resulted in improvements in scar outcomes but the risk of negative impact of scarring on a patient remains high. Scar tissue is described as containing thicker collagen bundles or having more parallel alignment of collagen fibers relative to normal skin. Assessment of scar reduction following treatment is limited by an absence of reliable and sensitive tools to quantitate collagen fiber alignment.

Aims

This study aims to highlight the limitations of Fast Fourier Transformation (FFT) and OrientationJ Coherency measurement in the quantitation of fiber alignment. This study also aims to explore the behaviour of the OrientationJ plugin for ImageJ through the analysis of computer generated test images and polarized light microscopy images of normal human skin and human burn scar. Through collaboration with a mathematician this study aims to propose an alternative mathematical interpretation of OrientationJ distribution tool as an alternative method for measuring fibre alignment.

Methods

Computer generated images (n=30) were designed using Adobe Illustrator with variations in line angle, curvature and thickness. Histological slides were prepared from normal human skin (n=9) and human burn scar tissue (n=20). Tissue was fixed in 4% paraformaldehyde for 24hours at 4°c, paraffin embedded, cut to 5µm and stained with picrosirius red. Slides were imaged using an Olympus IX81 inverted microscope (Olympus, Tokyo, Japan). Polarization filters were orientated with maximum cross polarisation and confirmed using a control tissue slide. Images were acquired with a 10x objective (NA, Olympus), Nikon DS-2Mv camera (Nikon, Tokyo, Japan)and NIS Elements software (V4.3, 1600x1200, Nikon, Tokyo, Japan). Images were analysed using ImageJ (v1.51f 64bit) FFT, OrientationJ Coherency and OrientationJ Distribution tools.

Results

Computer generated test images demonstrated that orientation analysis is influenced by image format, magnification at image acquisition, and orientation of the structure within the frame. Test images highlighted the fundamental difficultly in measuring fiber alignment using pixels, which are orientated at 0° and 90°.

Analysis of human control and scar tissue using FFT and OrientationJ Coherency tool resulted in an underestimation of parallel fiber alignment where several dominant directions were observed . This underestimation was sufficient in computer generated images to give a near perfect disordered value to a perfectly ordered image. OrientationJ Distribution tool was not subject to this limitation.

Conclusion

Computer generated test images are useful in determining whether image analysis software is appropriate for orientation analysis and can assist with experimental design for optimal image acquisition. OrientationJ Coherency and FFT underestimate parallel fiber alignment in samples with two prominent orientations. OrientationJ Distribution is an alternative measurement tool for fiber alignment.